In re Application of YANG
Confirmation No: 9213
Application No. 10/640,989
Examiner: Afremova, Vera

REMARKS

Claims 1-24 are pending in the application. Claims 2-7, 10, 11 and 14-24 are withdrawn from consideration as being directed to non-elected subject matter. Applicants hereby reserve the right to pursue the subject matter of the canceled claims in one or more divisional patent applications.

Claims 12 and 13 have been amended to recite an "insulin producing cell." No new matter has been added by virtue of this amendment and their entry is respectfully requested.

Claim Objections

Claim 1 is objected to as the Latin phrase "in vitro" should be italicized. Applicants have corrected this informality.

Claim Rejections Under 35 U.S.C. § 112

Claims 8 and 9 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have amended the claims to recite "cultured." In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejection Under 35 U.S.C. § 102

Claims 1, 8 and 9 are rejected under 35 U.S.C. § 102(e) as being anticipated by Ramiya *et al.* (2002, U.S. Patent Application Publication 2002/0182728; reference A).

Applicants respectfully traverse.

Applicants describe an insulin producing cell. Isolated from an *in vitro* culture of human bone marrow. Applicants teach that these cells produce the actual insulin molecule and do not just express insulin mRNA. See, for example, page 8, lines 20-31:

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> To determine if the differentiated BM-derived cells actually synthesized endocrine hormone proteins, cells were first detached by 0.25% of trypsin-EDTA, then incubated in Medium C in a 10 mL test tube in an incubator for two hours before embedding in a paraffin cell block. The presence of the endocrine cell hormones insulin and glucagon in trans-differentiated BM-derived cells was detected by immunocytochemical staining. The paraffin block sections were stained with either hematoxylin-eosin (H&E) stains for morphologic evaluation or probed with the primary antibodies against the endocrine hormones insulin (polyclonal guinea pig anti-rat, DAKO, Carpinteria, CA) and glucagon (DAKO, Carpinteria, CA). Antibodies to human albumin and cytokeratin CK19 were used as negative controls. Human pancreas was used as a positive control. The results showed pancreatic endocrine hormone (insulin and glucagon) production after 2 weeks of continued growth under high glucose conditions. (Emphasis added).

Applicants also detect the secreted insulin using an ELISA. See, for example, page 12, lines 1-11:

> Human insulin ELISA. Differentiated HBMDS cells were cultured in the presence or absence of 10-mM nicotinamide, or exendin 4, or both for 5-7 days in RPMI 1640 containing 5% FBS, and 5.5 mM glucose after the cells were confirmed to express insulin genes by RT-PCR. The cells were switched to serum-free medium containing 0.5% bovine serum albumin (BSA) for 12 hrs, washed twice with PBS, then stimulated by the addition of 17.5 mM additional glucose (final concentration of 23 mM) for various times. The culture media were collected and frozen at -70°C until assayed for insulin release. The serum-free culture medium containing 0.5% BSA was used as a control for secreted insulin measurements. Insulin release was detected by using a human insulin ELISA kit (ALPCO Diagnostics, Windham, NH) with sensitivity of 0.15 μU/ml following the manufacturer's protocols. This assay does not detect proinsulin.

Ramiya *et al.*, do not teach an insulin **producing** cell isolated from human bone marrow cells. Applicants submit that expression of insulin mRNA is not the same as producing the hormone. Ramiya *et al.*, do not teach or suggest that these cells actually produce any secreted insulin hormone. See, for example, page 6, paragraph [0059]:

> [0059] Upon receipt of the HSCs, they were cultured in basal medium (Table 1A) for two days. After two days of rest, the cells were split into groups and cultured in various factors. After 14 and 45 days,

> RNA was extracted for RT-PCR/Southern blot analyses to determine the expression of genes relevant to pancreas organogenesis, and not known to be involved in hematopoietic or mesenchymal pathways of differentiation. These genes include Isl-1, Pdx-1, Pax -4, Pax-6, Glut-2 and insulin. Human bone marrow-derived $CD34^+$ stem cells treated with basal medium containing various factors (Table 1B) for 2 weeks expressed mRNAs for Isl-1, Pax-6, CK-19 along with CD34 (FIG. 1). The continuous culture of cells up to 45 days resulted in the **expression** of mRNA for insulin and maintenance of CK19 (FIG. 1). (Emphasis added).

Black *et al.*, pre-treat human bone marrow cells with an endodermal/neuronal precursor inducing compound to produce a nestin-positive endodermal/neuronal precursor cell. The method requires the pre-differentiation of cells. Furthermore, Black *et al.*, do not teach or disclose what the compound is, the concentration of the compound, the length of culture of the compound with the cells, and any amounts of insulin produced. That is, Black *et al*, is non-enabling. Black *et al*, refer to marrow stromal cells as "stem cell-like precursors of osteocytes, chondrocytes, adipocytes and various other cell types and which are isolated from bone marrow by their ability adhere to plastic dishes." (Paragraph [0078]). In summary, Black *et al.*, do not teach or disclose the instant invention.

In view thereof, these claims are allowable under 35 U.S.C. § 102. Applicants respectfully request reconsideration and withdrawal of the instant invention.

Claim Rejections Under 35 U.S.C. § 103

Claims 1, 8, and 9 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Wang *et al.*, (1998, *Chinese Medical Journal English Edition* 111:899-902: reference U) taken in view of Kuznetsov *et al.* (1997, *Journal of Bone and Mineral Research* 12:1335-1347; reference V).

Applicants respectfully traverse.

Wang *et al.*, do not teach the isolation of a human bone marrow stem cell that produces insulin. Rather Wang *et al.*, transduce an insulin gene into the cell. Cells, which do not contain

the transfected insulin gene do not produce insulin, no matter how long in culture. (See, Table, page 900, column 2).

Kuzetsnov *et al.*, do not teach an insulin producing cell from bone marrow. Rather, Kuzetsnov *et al.*, are concerned with osteogenic cells. Even assuming arguendo, that one of ordinary skill in the art transduces the cells of Kuzetsnov *et al.*, with the insulin gene of Wang *et al.*, one of ordinary skill in the art would not arrive at the instant invention, i.e. "an insulin-producing cell isolated from an *in vitro* culture of bone marrow cells obtained from a human subject." These cited references teach away from an insulin producing cell which has **not** been transformed with a vector encoding an insulin gene. These cited references teach away from an insulin producing cell which has **not** been transformed with a vector encoding an insulin gene and the only way that a cell would produce insulin would be the introduction of the insulin gene into a cell in order to produce insulin. That is, for a fibroblast to produce insulin, the fibroblast must be transfected with an insulin gene. Furthermore, neither of these references teach human bone marrow stem cells. Neither of these references provide the motivation for an insulin producing cell of the instant invention.

In view thereof, the instant claims are allowable under 35 U.S.C. § 103(a). Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 1, 8, 9, 12 and 13 are rejected under 35 U.S.C. § 103(a) as being unpatentable over either Ramiya *et al.* (reference A) or Black et al (reference B) taken in view of Boyse *et al.* (1991, U.S. Patent 5,004,681; reference C), Polovina (1996, U.S. Patent 5,580,714; reference D), and Gianni (1997, U.S. Patent 5,649,904; reference E).

Applicants respectfully traverse.

As discussed above, Ramiya et al., do not teach an insulin **producing** cell that has been frozen. Black et al., do not teach or disclose a frozen insulin producing cell. With respect to Gianni, Polovina and Boyse et al., neither of these references teach or disclose a frozen insulin

producing cell that is obtained from human bone marrow cells as taught by applicants. One of ordinary skill in the art would not be motivated to freeze an insulin producing cell as none of these references whether the cell would actually still produce insulin after it has been frozen.

In view thereof, the claims are allowable over the cited references under 35 U.S.C. § 103. Applicants respectfully request reconsideration and withdrawal of the instant invention.

Claims 1, 8, 9, 12, and 13 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Wang et al., taken in view of Kuznetsov et al., as applied to claims 1, 8 and 9 above, and further in view of Boyse et al., Polovina, and Gianni.

As discussed above, Wang et al., do not teach the isolation of a human bone marrow stem cell that produces insulin. Rather Wang *et al.*, transduce an insulin gene into the cell. Cells, which do not contain the transfected insulin gene do not produce insulin, no matter how long in culture. (See, Table, page 900, column 2).

Kuzetsnov *et al.*, do not teach an insulin producing cell from bone marrow. Rather, Kuzetsnov *et al.*, are concerned with osteogenic cells that were isolated from human foreskin. One of ordinary skill in the art would not arrive at the instant invention even if these cells were transduced with the insulin gene. Applicants teach "an insulin-producing cell isolated from an *in vitro* culture of bone marrow cells obtained from a human subject." The foreskin fibroblasts described in Kuzetsnov *et al.*, are committed to the osteogenic lineage and would not motivate one of ordinary skill in the art to transduce these cells with an insulin gene. These cited references teach away from an insulin producing cell which has **not** been transformed with a vector encoding an insulin gene and the only way that a cell would produce insulin would be the introduction of the insulin gene into a cell in order to produce insulin. That is, for a fibroblast to produce insulin, the fibroblast must be transfected with an insulin gene. Furthermore, neither of these reference teach the freezing of the cells of the instant invention.

With respect to Gianni, Polovina and Boyse *et al.*, neither of these references teach or disclose a frozen insulin producing cell that is obtained from human bone marrow cells as taught by applicants. Since Wang *et al*, and Kuzestnove *et al.*, teach away from the instant invention, one of ordinary skill in the art would not be motivated to combine these references with Gianni, Polovina and Boyse *et al*. Applicants, therefore, submit that neither of these references alone or in combination teach the instant invention.

In view thereof, Applicants submit that the claims are allowable over the cited references under 35 U.S.C. § 103. Applicants respectfully request reconsideration and removal of the instant rejection.

Applicants have made every effort to present claims which distinguish over the cited art, and it is believed that all claims are now in condition for allowance. However, Applicants request that the Examiner call the undersigned (direct line 561-671-3666) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 11, 8, 8, 12 and13 is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no extensions of time are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

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Respectfully submitted,

AKERMAN SENTERFITT

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